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Incident type 2 diabetes is associated with HDL, but not with its anti-oxidant constituent - paraoxonase-1: The PREVEND study

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ABSTRACT

Objective. High-density lipoprotein cholesterol (HDL-C) is an established risk marker for cardiovascular disease and consistently associated with type 2 diabetes risk. Serum paraoxonase-1 (PON-1) - an anti-oxidant constituent of HDL - is inversely associated with cardiovascular disease risk, but its relationship with incident type 2 diabetes is uncertain. We aimed to investigate the prospective association between PON-1 and type 2 diabetes risk.

Methods. PON-1 was measured as its arylesterase activity at baseline in the Prevention of Renal and Vascular End-stage Disease (PREVEND) prospective study of 5,947 predominantly Caucasian participants aged 29-76 years with no pre-existing diabetes, that recorded 500 type 2 diabetes cases during a median follow-up of 11.2 years.

Results. Serum PON-1 was positively correlated with HDL-C (r=0.17; $P < 0.001$). In analyses adjusted for conventional diabetes risk factors, the hazard ratio (95% CI) for type 2 diabetes per 1 standard deviation increase in PON-1 was 1.07 (0.98 to 1.18; P=0.13), which remained non-significant (1.02 (0.93 to 1.12) P=0.65) after additional adjustment for potential confounders. The association was unchanged on further adjustment for HDL-C (1.05 (0.96 to 1.15; P=0.29). However, in subsidiary analyses in the same set of participants, serum HDL-C concentration was inversely and independently associated with risk of type 2 diabetes.

Conclusions. Incident type 2 diabetes is associated with HDL cholesterol but not with its anti-oxidant constituent - PON-1 - in a large cohort of apparently healthy men and women. The current data question the importance of PON-1 activity for the development of diabetes.

Keywords: Paraoxonase; HDL cholesterol; antioxidant; risk factors; type 2 diabetes

Nonstandard Abbreviations and Acronyms

BMI body mass index
CI confidence interval
CKD-EPI Chronic Kidney Disease Epidemiology Collaboration
CVD cardiovascular disease
eGFR estimated glomerular filtration rate
HDL-C high-density lipoprotein cholesterol
HOMA-IR homeostasis model assessment of insulin resistance
HR hazard ratio
PON-1 paraoxonase-1
PREVEND Prevention of Renal and Vascular End-stage Disease
SD standard deviation
SBP systolic blood pressure
UAE urinary albumin excretion

1. Introduction

High-density lipoprotein cholesterol (HDL-C) is an established risk marker for atherosclerotic cardiovascular disease (CVD).(1) HDL-C levels have also been shown to be consistently associated with risk of type 2 diabetes.(2-4) HDL is known to exert antioxidant and anti-inflammatory properties.(5; 6) Paraoxonase-1 (PON-1), which is synthesized by the liver,(7) is a HDL-bound esterase enzyme and has well-established antioxidant and anti-inflammatory properties.(5; 6; 8; 9) HDL essentially provides a vector that facilitates the secretion of PON-1 by the liver(10) and also creates a hydrophobic environment which could be important for PON-1 function.(11) In our recent prospective cohort analysis and meta-analysis of six population-based prospective studies, we have shown that serum PON-1 activity is inversely associated with CVD risk, but this association is at least in part dependent on HDL-C levels.(12) Evidence suggests that the ability of the HDL fraction to inhibit oxidative modification of low-density lipoproteins and its anti-atherogenic and cardioprotective effects are to a considerable extent explained by PON-1 activity.(6; 13; 14) Oxidative stress is known to play a central role in the aetiopathogenesis of diabetes(15-17) and therefore HDL may contribute to the pathophysiology of type 2 diabetes via its antioxidant effects. Patients with type 2 diabetes have also been consistently shown to exhibit low circulating PON-activity, albeit in conjunction with low HDL-C.(18-20) Taking together all the evidence, we hypothesized that PON-1 activity may be linked to type 2 diabetes risk. Data showing the relationship between serum PON-1 and type 2 diabetes are sparse and have largely been based on animal models and cross-sectional or case-control study designs.(19; 21; 22) Though a consistent body of observational evidence shows HDL to be prospectively linked to the development of type 2 diabetes,(2-4) our search of the literature

showed that the prospective relationship between serum PON-1 activity and type 2 diabetes has not been previously investigated. It is therefore not clear whether increased PON-1 activity reduces the risk of type 2 diabetes among apparently healthy individuals. Our primary objective was, therefore, to evaluate in detail the nature and magnitude of the prospective association between serum PON-1 activity and risk of type 2 diabetes in a population-based sample of 5,947 participants free from pre-existing diabetes at baseline. A secondary objective was to investigate if the expected inverse association of serum HDL-C concentration with risk of type 2 diabetes was independent of PON-1 activity in the same set of participants.

2. Material and methods

This report was conducted according to STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for reporting observational studies in epidemiology (**Appendix A**).(23)

2.1. Participants and study settings

The study population is based on the Prevention of Renal and Vascular End-stage Disease (PREVEND) study, an observational, general population-based prospective cohort study which began in 1997, with participants (age range 28-75 years) drawn from inhabitants living in the city of Groningen in The Netherlands. The PREVEND study was designed to investigate the natural course of urinary albumin excretion and its relationship to renal disease and cardiometabolic outcomes. The description of the study design and recruitment processes has been described in detail previously.(24) The actual baseline cohort consisting of 8,592 participants had baseline measurements performed between 1997 and 1998. For the present analyses, we excluded

participants with a prevalent history of diabetes [defined by either a self-report of physician diagnosis or screening at first visits (1996–1997)], leaving a final cohort of 5,947 participants who were free of baseline diabetes with non-missing information on serum PON-1 activity, several diabetes risk markers, and incident type 2 diabetes. The local medical ethics committee of the University Medical Center Groningen approved the PREVEND study and which was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent which was documented in a consent form approved by the medical ethics committee.

2.2. Risk factor assessment

During two outpatient visits by study participants, baseline data on demographics, physical measurements (including anthropometrics), and cardiovascular and metabolic risk factors were assessed during five rounds of screening from 1997 to 1998 until January 1st, 2011. Information on use of medications was collected via data from pharmacy registries of all community pharmacies in the city of Groningen. Hypertension was defined by self-reported physician diagnosis, use of antihypertensive medication, or blood pressure ≥ 140/90mmHg. After an overnight fast and 15 minutes of rest, venous blood was obtained from participants. All blood samples were taken between 8.00 and 10.00 am. Plasma samples were prepared by centrifugation at 4 ºC.

Sera were stored at -80 ºC until analysis. Serum PON-1 enzymatic activity was measured as its arylesterase activity, i.e. as the rate of hydrolysis of phenyl acetate into phenol, as described previously.(25) The inter-assay CV was 8%. Arylesterase activity, measured with this assay, is positively correlated PON-1 enzymatic activity toward paraoxon.(26) HDL-C was measured by a homogeneous method (direct HDL, Aeroset System; Abbott Laboratories, Abbott

Park, Illinois). Glucose, total cholesterol, triglycerides, total bilirubin, fasting insulin, creatinine, and cystatin C were measured using standard methods described previously.(27-29) Urinary albumin excretion (UAE) was estimated as the mean of two 24-hour urine collections. Estimated glomerular filtration rate (eGFR), was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation.(30) Insulin resistance was estimated according to the homeostasis model assessment of insulin resistance (HOMA-IR) (the product of fasting glucose [mmol/l] and insulin [units/ml] divided by the constant 22.5(31)).

2.3. Endpoint ascertainment

New onset type 2 diabetes was ascertained if one or more of the following criteria were met during follow-up beyond 3 months after baseline: (i) fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL); (ii) random sample plasma glucose ≥ 11.1 mmol/L (200 mg/dL); (iii) self-report of a physician diagnosis; and (iv) initiation of glucose lowering medication use retrieved from a central pharmacy registry.

2.4. Statistical analyses

Variables that were skewed (e.g., triglycerides, creatinine, UAE, fasting insulin, and HOMA-IR) were natural logarithm ($\log_e$) transformed to achieve approximately normal distributions. Baseline characteristics of participants were summarized using descriptive analyses. To assess the cross-sectional associations of serum PON-1 activity with risk markers for type 2 diabetes, we estimated partial correlation coefficients (adjusted for age and sex). Cox proportional hazards models after confirmation of assumptions of proportionality of hazards,(32) were used to assess

the association between serum PON-1 activity and type 2 diabetes risk. Hazard ratios (HRs) were calculated per 1 standard deviation (SD) higher serum PON-1 values and by quintiles defined according to the baseline distribution of serum PON-1 values. To assess the independence of the association, HRs were calculated with progressive adjustment for age and sex; other established risk factors for type 2 diabetes [fasting glucose, body mass index (BMI), SBP, smoking status, alcohol consumption, and parental history of diabetes], potential confounders [triglycerides, eGFR, UAE, and HOMA-IR], and finally HDL-C. Effect modification by individual characteristics, such as age, sex, and other diabetes risk markers was assessed using formal tests of interaction. We conducted sensitivity analyses employing the use of complex survey design analyses,(33) taking into account that the PREVEND cohort is oversampled for subjects with higher albuminuria levels, thereby enabling the results to be extrapolated to the general population. All statistical analyses were conducted using Stata version 14 (Stata Corp, College Station, Texas).

3. Results

3.1. Baseline characteristics and correlates of paraoxonase-1 activity

Data were available for 5,947 participants without a known history of diabetes at baseline. The mean age of overall participants at baseline was 49 (SD 12) years and 50% were women (**Table 1**). The mean (SD) for PON-1 was 56.0 (17.8) U/L. PON-1 values were weakly correlated with physical measures (BMI and waist-to-hip ratio), as well as several lipid, metabolic, and renal markers. The strongest correlations were observed for HDL-C (r = 0.17) and total cholesterol (r = 0.11). Baseline PON-1 values were lower by in males compared with females (**Table 2**).

3.2. Paraoxonase-1 activity and risk of incident type 2 diabetes

During a median (interquartile range) follow-up of 11.2 (7.2-12.0) years, 500 incident type 2 diabetes cases were recorded. The HR for type 2 diabetes per 1 SD increase in PON-1 was 0.99 (95% CI, 0.90 to 1.08; *P*=0.79) in age- and sex-adjusted analyses, which remained non-significant in analyses adjusted for established diabetes risk factors 1.07 (95% CI, 0.958 to 1.18; *P*=0.13), and additional adjustment for $\log_e$ triglycerides, eGFR, $\log_e$ UAE, and $\log_e$ HOMA-IR 1.02 (95% CI, 0.93 to 1.12; *P*=0.65). This association remained unaltered after additional adjustment for HDL-C 1.05 (95% CI, 0.96 to 1.15; *P*=0.29). The null associations were also maintained in analyses by quintiles of the baseline distribution of PON-1 values (**Table 3**). The associations did not vary significantly by levels or categories of several clinically relevant individual characteristics (**Figure**). To put our findings into context, direct comparisons were made to the associations of HDL-C with type 2 diabetes events in the same set of participants. As consistently demonstrated in previous prospective studies, serum HDL-C concentration was strongly inversely and independently associated with risk of type 2 diabetes. The association was also independent of serum PON-1 activity (**Table 4**). All results were essentially similar when design-based Cox regression analyses were performed (**Appendices B and C**).

4. Discussion

4.1. Key findings

In the PREVEND prospective cohort comprising apparent healthy men and women without a history of diabetes at baseline, we observed a modestly positive but highly significant correlation between PON-1 activity and HDL-C. There was no evidence of an association between baseline PON-1 values and risk of future type 2 diabetes. The null associations remained consistent across several clinically relevant subgroups. Importantly however, our data showed an inverse association between HDL-C and type 2 diabetes risk in the same set of participants in analyses adjusted for several established risk factors in agreement with several previous studies,(2-4). Of interest, this association was independent of serum PON-1 activity.

4.2. Possible explanations for findings

Results from our correlational analysis showed a weak but highly significant association between PON-1 and HDL-C. These findings may seem unexpected given the close physiological relationship existing between circulating PON-1 activity and HDL; however, similar as well as diverging estimates have been reported in previous studies(34-36) and this may also reflect the heterogeneous nature of HDL particle size, number, and composition. For example, one study found that PON-1 activity on total HDL was almost completely expressed by the HDL_3 subspecies fraction.(37) In line with consistent observational evidence which show that low levels of HDL-C are associated with an increased risk of type 2 diabetes, accruing evidence suggests that HDL may be directly involved in the pathogenesis of type 2 diabetes through direct effects on plasma glucose levels.(38) In both human and animal studies, HDL stimulates

pancreatic β-cell function and modulates glucose uptake in skeletal muscle.(39-42) Nonetheless, recent genetic evidence suggests that low HDL-C may not be causally linked to the risk of type 2 diabetes,(43) suggesting that the observational associations could be due to reverse causation and/or residual confounding. PON-1 is an important component of HDL (which acts as its carrier and site of action(20; 44; 45)) with well recognized antioxidant properties. The ability of HDL particles to metabolize lipid peroxides and prevent low-density lipoprotein oxidation has been largely attributed to PON-1 activity.(14; 46) Given the broad body of evidence that demonstrates oxidative stress to play a central role in the development of diabetes, (15; 16) and the low PON-1 activity observed in people with type 2 diabetes,(18; 19) our findings of a null association are surprising. However, β-cell function has been shown to relate positively to HDL anti-oxidative functionality only in subjects with established type 2 diabetes, but not in subjects with normal fasting glucose or impaired fasting glucose.(47) The current findings of a null association between PON-1 and type 2 diabetes association are in contrast to the inverse association demonstrated between HDL-C and type 2 diabetes.(2-4) Previous studies have however consistently demonstrated HDL-C and PON-1 to be each inversely associated with CVD risk.(1; 12) The lack of evidence of an association between PON-1 and type 2 diabetes may reflect important pathophysiologic differences existing between HDL-C as an estimate of the cholesterol content of all HDL particles combined, as well as of HDL particle numbers, and PON-1 in the pathogenesis of diabetes. Though PON-1 is a determinant of serum concentrations of HDL-C,(34) there have been suggestions that other factors may modulate the relationship between PON-1 and HDL(34) and which include the inability of PON-1 to exert its full antioxidant potential.(11) The weak correlation between PON-1 and HDL-C in the PREVEND population may also explain their divergent associations with type 2 diabetes. Further

characterization of HDL subfractions may help elucidate these findings. In addition, given the absence of previous studies conducted on the topic, larger-scale studies are warranted to confirm or refute these findings.

4.3. Strengths and limitations

The strengths of the present study merit consideration. To our knowledge, it is the first comprehensive investigation of the observational epidemiological prospective association between PON-1, using an established method to measure its activity, and type 2 diabetes risk in the general population. Participants in the PREVEND study were recruited from a predominantly Caucasian North European population, were well characterized, involved a high response and follow-up rates and had been prospectively monitored using established databases for type 2 diabetes endpoints. Our analysis was based on a large prospective population-based cohort study, therefore had high statistical power to examine the associations in greater detail, including subgroup analyses at different levels or categories of diabetes risk factors. Individuals with prevalent diabetes at baseline were excluded from the analyses, limiting any possibilities of reverse-causation bias. The study had complete measurements on a panel of lifestyle and biological markers permitting adequate adjustment for several potential confounders. All blood samples were taken from study participants in the morning between 8.00 and 10.00 am; therefore, the likelihood of diurnal variation in blood analytes including PON-1 was minimal. The reliability of the data was also confirmed by our ability to replicate the independent association of HDL-C with type 2 diabetes.(2-4) Several limitations also deserve mention. Although we accounted for a number of potential confounders including key clinical characteristics, there is still a potential for residual confounding due to errors in measurements of

risk marker and other unmeasured confounders (such as physical activity). We only had a one-time measurement of PON-1 activity, therefore, we could not correct for its within-individual variation in serum over time. However, it should be realized that analysis using only baseline or one-time measurements of an exposure in epidemiological studies could considerably underestimate the true strength of any aetiological association between the exposure and disease outcome (i.e. "regression dilution bias"(48)). If the intra-individual variability of the exposure is taken into account, this results in strengthening of any associations that existed for single baseline measurements of the exposure.(49) Finally, given the suggested interpopulation differences in serum paraoxonase specific activity which have been demonstrated in healthy populations(50) and the fact that our analyses were based on a predominantly white-European population, the findings may not be generalizable to individuals of different ethnicities.

4.4. Conclusion

Incident type 2 diabetes is associated with HDL cholesterol but not with its anti-oxidant constituent - PON-1 - in a large cohort of apparently healthy Caucasian men and women. The current data suggest that there may be important pathophysiologic differences between HDL-C and PON-1-1 in the pathogenesis of diabetes. The relationship between PON-1 and HDL warrants further investigation.

Author Contributions

SKK, LMK, SJLB, RWJ and RPFD conceived and designed the study. SJLB, LMK, RWJ and RPFD acquired data. SKK analyzed and interpreted the data. SKK drafted the manuscript. SKK, LMK, SJLB, RWJ and RPFD critically revised the manuscript for important intellectual content. RPFD supervised the study. S.K.K. is the guarantor of this work, and as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

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Conflict of interest

The authors declare that there is no duality of interest associated with this manuscript.

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Table 1. Baseline participant characteristics overall and according to the development of incident type 2 diabetes

	Overall (N=5,947) Mean (SD) or median (IQR) or n (%)	**Without incident type 2 diabetes (N=5,447) Mean (SD) median (IQR) or n (%)**	**With incident type 2 diabetes (N=500) Mean (SD) or median (IQR) or n (%)**	***P*-value**
Serum paraoxonase-1 (U/L)	56.0 (17.8)	56.1 (17.9)	55.0 (17.2)	0.167
Questionnaire				
Males	2,953 (49.7)	2,653 (48.7)	300 (60.0)	< 0.001
Age at survey (years)	49 (12)	49 (12)	55 (10)	< 0.0001
Smoking				
Current	1,907 (32.1)	1,741 (32.0)	166 (33.2)	
Former	2,228 (37.5)	2,026 (37.2)	202 (40.4)	0.109
Never	1,812 (30.5)	1,680 (30.8)	132 (26.4)	
Alcohol consumers	4,545 (76.4)	4,193 (77.0)	352 (70.4)	0.001
History of hypertension	1,817 (30.6)	1,518 (27.9)	299 (59.8)	< 0.001
Parental history of diabetes	861 (14.5)	738 (13.6)	123 (24.6)	< 0.001
Physical measurements				
BMI (kg/m^2)	26 (4)	26 (4)	29 (5)	< 0.0001
WHR	0.88 (0.09)	0.87 (0.09)	0.94 (0.08)	< 0.0001
SBP (mmHg)	128 (19)	126 (19)	140 (20)	< 0.0001
DBP (mmHg)	74 (10)	73 (9)	79 (9)	< 0.0001
Lipid markers				
Total cholesterol (mmol/l)	5.61 (1.11)	5.57 (1.10)	6.01 (1.15)	< 0.0001
HDL-C (mmol/l)	1.34 (0.40)	1.36 (0.40)	1.12 (0.31)	< 0.0001
Triglycerides (mmol/l)	1.13 (0.83-1.64)	1.09 (0.81-1.57)	1.66 (1.18-2.41)	< 0.0001
Metabolic and renal markers				
Glucose (mmol/l)	4.70 (0.66)	4.63 (0.57)	5.55 (0.94)	< 0.0001
Fasting insulin (units/ml)	7.8 (5.5-11.5)	7.5 (5.3-10.9)	13.0 (8.9-19.6)	< 0.0001
HOMA-IR	1.60 (1.08-2.48)	1.53 (1.05-2.29)	3.13 (2.14-5.05)	< 0.0001
Creatinine (µmol/l)	82 (74-92)	82 (73-91)	86 (76-96)	< 0.0001
Cystatine C (mg/dl)	0.79 (0.20)	0.79 (0.19)	0.84 (0.21)	< 0.0001
eGFR ($ml/min/1.73\ m^2$)	95.7 (16.6)	96.1 (16.4)	91.1 (17.3)	< 0.0001
UAE (mg/24 hours)	9.04 (6.21-15.59)	8.78 (6.14-14.61)	13.59 (7.60-32.28)	< 0.0001

BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IQR, interquartile range; SBP, systolic blood pressure; UAE, urinary albumin excretion; WHR, waist-to-hip ratio

Table 2. Cross-sectional correlates of paraoxonase-1 activity

	Partial correlation r (95% CI)†	Absolute difference (95% CI) in paraoxonase-1 values per 1 SD higher or compared to reference category of correlate‡
Serum paraoxonase-1 (U/L)	-	-
Sex		
Female	-	Ref
Male	-	-2.29 (-3.20, -1.39)***
Questionnaire		
Age at survey (years)	-0.07 (-0.09, -0.04)***	-1.23 (-1.68, -0.77)***
Smoking status		
Non-smokers	-	Ref
Current and former smokers	-	-0.38 (-1.36, 0.61)
Alcohol consumption		
Non-consumers	-	Ref
Current consumers	-	3.33 (2.25, 4.40)***
History of hypertension		
No	-	Ref
Yes	-	0.49 (-0.61, 1.60)
Parental history of diabetes		
No		Ref
Yes		-0.87 (-2.15, 0.41)
Physical measurements		
BMI (kg/m^2)	-0.01 (-0.04, 0.01)*	-0.20 (-0.67, 0.27)
WHR	-0.02 (-0.04, 0.01)***	-0.47 (-1.09, 0.15)
SBP (mmHg)	0.05 (0.03, 0.08)	1.05 (0.53, 1.58)***
DBP (mmHg)	0.05 (0.02, 0.07)	0.99 (0.48, 1.51)**
Lipid markers		
Total cholesterol (mmol/l)	0.11 (0.09, 0.14)***	2.15 (1.68, 2.63)***
HDL-C (mmol/l)	0.17 (0.14, 0.19)***	3.28 (2.79, 3.77)***
Log triglycerides (mmol/l)	0.08 (0.05, 0.10)**	1.40 (0.93, 1.86)***
Metabolic and renal markers		
Glucose (mmol/l)	-0.04 (-0.06, -0.01)***	-0.69 (-1.17, -0.21)*
Log$_e$ Fasting insulin (units/ml)	0.03 (0.00, 0.06)	0.54 (0.09, 1.00)*
Log$_e$ HOMA-IR	0.02 (-0.01, 0.05)	0.36 (-0.10, 0.82)
Log$_e$ creatinine (µmol/l)	0.01 (-0.01, 0.04)*	0.32 (-0.23, 0.87)
Cystatine C (mg/dl)	-0.06 (-0.09, -0.04)***	-1.19 (-1.67, -0.71)***
eGFR (ml/min/1.73 m^2)	0.02 (-0.00, 0.05)***	0.50 (-0.09, 1.09)
Log$_e$ UAE (mg/24 hours)	0.02 (-0.01, 0.05)	0.37 (-0.09, 0.84)

BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; Ref, reference; SD, standard deviation; SBP, systolic blood pressure; UAE, urinary albumin excretion; WHR, waist-to-hip ratio

Asterisks indicate the level of statistical significance: *, p<0.05; **, p<0.01; ***, p<0.001; †, Pearson correlation coefficients between paraoxonase-1 and the row variables; ‡, Absolute change in paraoxonase-1 values per 1 SD increase in the row variable (or for categorical variables, the absolute difference in mean paraoxonase-1 values for the category versus the reference) adjusted for age and sex;

Table 3. Association of serum paraoxonase-1 activity with incident type 2 diabetes

PON-1 activity (U/L)	Events/ Total	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Per 1 SD increase	500 / 5,947	0.99 (0.90 to 1.08)	0.79	1.07 (0.98 to 1.18)	0.13	1.02 (0.93 to 1.12)	0.65	1.05 (0.96 to 1.15)	0.29
Q1 (0.84-41-35)	104 / 1,190	ref		ref		ref		ref	
Q2 (41.36-49.67)	102 / 1,189	1.01 (0.77 to 1.33)	0.92	1.40 (1.05 to 1.87)	0.02	1.25 (0.94 to 1.66)	0.13	1.28 (0.96 to 1.71)	0.09
Q3 (49.68-57.41)	106 / 1,190	1.11 (0.84 to 1.45)	0.46	1.35 (1.01 to 1.79)	0.04	1.24 (0.94 to 1.65)	0.13	1.31 (0.99 to 1.75)	0.06
Q4 (57.42-68.96)	104 / 1,189	1.09 (0.83 to 1.43)	0.54	1.45 (1.08 to 1.93)	0.01	1.24 (0.93 to 1.65)	0.15	1.33 (1.00 to 1.78)	0.05
Q5 (> 68.96)	84 / 1,189	0.92 (0.69 to 1.23)	0.56	1.35 (0.99 to 1.83)	0.06	1.15 (0.85 to 1.56)	0.36	1.26 (0.93 to 1.72)	0.14

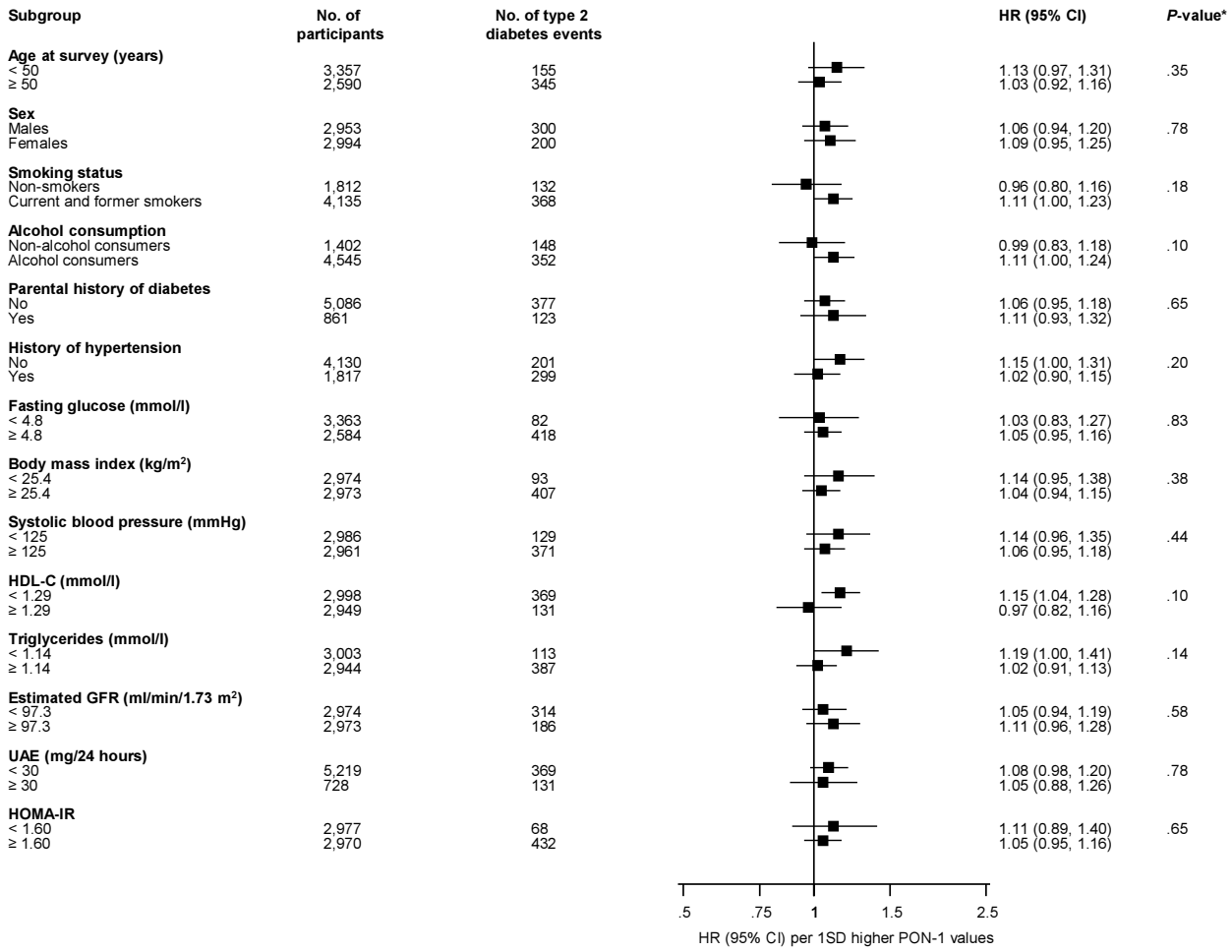
PON-1, paraoxonase-1; Q, quintile; SD, standard deviation
Model 1: Age and sex
Model 2: Model 1 plus fasting glucose, body mass index, systolic blood pressure, smoking status, alcohol consumption, and parental history of diabetes
Model 3: Model 2 plus $\log_e$ triglycerides, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation), $\log_e$ urinary albumin excretion and $\log_e$ homeostasis model assessment of insulin resistance
Model 4: Model 3 plus high-density lipoprotein cholesterol

Table 4. Association of serum high-density lipoprotein cholesterol with incident type 2 diabetes

Serum HDL-cholesterol (mmol/l)	Events/ Total	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Per 1 SD increase	500 / 5,947	0.47 (0.41 to 0.53)	< 0.01	0.63 (0.55 to 0.72)	< 0.01	0.81 (0.70 to 0.93)	0.004	0.79 (0.68 to 0.92)	0.002
Q1 (0.36-1.00)	200 / 1,237	ref		ref		ref		ref	
Q2 (1.01-1.19)	134 / 1,201	0.64 (0.51 to 0.80)	< 0.01	0.69 (0.54 to 0.87)	0.002	0.88 (0.69 to 1.12)	0.30	0.87 (0.68 to 1.11)	0.26
Q3 (1.20-1.39)	75 / 1,178	0.36 (0.27 to 0.47)	< 0.01	0.52 (0.39 to 0.68)	< 0.01	0.71 (0.53 to 0.96)	0.02	0.70 (0.52 to 0.94)	0.02
Q4 (1.40-1.65)	67 / 1,142	0.32 (0.24 to 0.42)	< 0.01	0.54 (0.40 to 0.73)	< 0.01	0.83 (0.60 to 1.15)	0.26	0.81 (0.58 to 1.13)	0.21
Q5 (≥ 1.66)	24 / 1,189	0.11 (0.07 to 0.17)	< 0.01	0.22 (0.14 to 0.35)	< 0.01	0.39 (0.24 to 0.63)	< 0.01	0.38 (0.23 to 0.61)	< 0.01

HDL, high-density lipoprotein; Q, quintile; SD, standard deviation
Model 1: Age and sex
Model 2: Model 1 plus fasting glucose, body mass index, systolic blood pressure, smoking status, alcohol consumption, and parental history of diabetes
Model 3: Model 2 plus $\log_e$ triglycerides, estimated glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation), $\log_e$ urinary albumin excretion, and $\log_e$ homeostasis model assessment of insulin resistance
Model 4: Model 3 plus paraoxonase-1

Figure. Hazard ratios for incident type 2 diabetes per 1 SD increase in baseline paraoxonase-1 values, by several participant level characteristics



Hazard ratios were adjusted for age, sex, fasting glucose, body mass index, systolic blood pressure, smoking status, alcohol consumption, and parental history of diabetes; CI, confidence interval (bars); Estimated GFR, glomerular filtration rate (as calculated using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation); HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HR, hazards ratio; SD, standard deviation; UAE, urinary albumin excretion; *, *P*-value for interaction; cut-offs used for fasting glucose, body mass index, systolic blood pressure, HDL-C, triglycerides, estimated GFR, and HOMA-IR are median values.